REMARKS

Favorable reconsideration of the subject application in view of the amendment above and comments below is respectfully requested.

Claims 1-12 and 15-20 are pending in the subject application. Claim 1 has been amended to correct a clerical error and to recite the time period for co-culturing. Support for the latter amendment is found at page 14. Claim 1 has further been amended to specify that the recombinant protein is recovered from the liquid co-culture. Support for this amendment is found at page 32, lined 16-19. Claim 10 has been amended to recite that the auxotroph has an impaired ability to grow in plant cell or root culture. This amendment is supported by Figure 4.

Accordingly, no new matter is added by these amendments to the claims.

I. Rejection of Claims 1-12 and 15-20 Under 35 U.S.C § 112, First Paragraph

Claims 1-12 and 15-20 stand rejected under 35 U.S.C § 112, first paragraph. The Examiner states that the specification does not enable a method of transforming monocotyledonous plant cells with Agrobacterium.

This rejection is respectfully traversed as follows.

The relevant prior art of record demonstrates that transient transformation of both monocots and dicots mediated by Agrobacteria was obtainable at the time of the invention. Also, the enclosed prior art reference (Narasimhulu et al., The Plant Cell, (1996) 8:873-886) demonstrates that it was known in the art that transient expression of monocots was readily obtainable, but that stable transformation of monocots was problematic ("the initial kinetics of gusA gene expression in maize cells are similar to

those shown in infected tobacco cells but that the presence of gusA mRNA in maize is highly transient suggests that the block to maize transformation involves T-DNA integration and not T-DNA entry into the cell or nuclear targeting.") (Abstract). Thus, it was recognized as early as 1996 that the delivery of T-DNA is the same for both monocots and dicots; the difference is that the T-DNA does not integrate into the chromosome of monocots. However, as repeatedly pointed out, the present invention does not involve integration of T-DNA into the plant chromosome, but instead is based on transient expression, which was demonstrated in the literature to be readily obtainable with both monocots and dicots.

The present invention, however, is not simply the transformation of monocots or dicots by Agrobacteria, and is not directed to the stable transformation of monocots or dicots. Instead, the present invention provides a method which can be applied to the large scale production of recombinant protein in plants that eliminates the need to establish stable transformation and stably transformed cell lines. The inventive method also advantageously eliminates the need to culture plant cells under conditions suitable for regeneration of plants or plant tissues, which requires weeks of culturing. Instead, the present invention requires only about one to about four days of co-culturing to obtain significant amounts of recombinant protein, as shown by example in the specification.

Accordingly, the rejection of claims 1-20 under 35 U.S.C. § 112, first paragraph is respectfully traversed.

II. Rejection of Claims 1-12 and 15-20 Under 35 U.S.C § 112, Second Paragraph

It is respectfully submitted that the amendments to the claims render the formal grounds of rejection moot.

III. Rejection of Claims 1-3, 5-7, 11, 16 and 18 Under 35 U.S.C § 102(b)

Claims 1-3, 5-7, 11, 16 and 18 are rejected under 35 U.S.C § 102(b) as being anticipated by Gomord et al. the Examiner states that the cited reference teaches culturing tobacco cells, inoculating the cells with Agrobacterium, followed by continued culturing and monitoring of the culture

The present invention is directed to methods of culturing and transiently transforming plant cells in liquid medium. Because the plants cells are maintained in liquid culture, it is possible to scale up the culture in a very short period of time (about 1one to about four days) to produce a biomass from which a large amount of recombinantly produced protein can be isolated. Specifically, in the claimed method recombinant proteins are recovered directly from cells in liquid culture without the need to obtain stable transformation of the plant cells.

In contrast, Gomord et al. discloses growing a 1 ml suspension of plant cells, which are then mixed with Agrobacterium and plated onto solid agar in Petri-dishes (static culture). This approach to transient expression is inherently not scalable because it is not conducted in a liquid volume. Gomord et al. cultured the plant cells in 150 mL of medium prior to transformation, but the transformation was not conducted in that volume or even in liquid suspension. Moreover, the "continued liquid suspension culture"

discussed by Gomord occurred one to two months after they had selected for stable transgenic cells.

The procedure used by Gomord et al. requires two to three weeks of selection, followed by growth of the selected cells. To obtain 100 L of cells that might be able to express the protein of interest using Gomord et al.'s takes about four months or more. Gomord explicitly states that the cell suspension of transgenic calli from which the protein is isolated is obtained in six to seven weeks after the selection; selection takes place on solid media for 2+ weeks after the co-culture with Agrobacterium (and after the Agrobacterium has been killed off with antibiotics). (p. 158).

The Examiner states that Gomord teaches transformation of plant cells and "final protein isolation two or three days later". However, this is not what the reference teaches. Gomord et al. used transient expression only in the 1 mL solid culture to provide for detection of protein expression with antibodies, i.e., as a control to demonstrate that expression had been achieved. Thus, Figure 2 of Gomord et al. is nothing more than a demonstration that the protein is in fact present during the solid culture transient expression stage that leads to stable transformation. Gomord et al. did not recover protein from a liquid co-culture, but merely separated proteins by electrophoresis and detected the recombinant protein using antibody. Gomord et al. do not teach protein recovery from liquid culture, and indeed, this reference teaches the necessity of obtaining stable transformation for the isolation of protein. In that regard, it is especially relevant that Gomord et al. cite Gomord's earlier work (Rayon et al, 1996), which discloses the use of 10 g of stably transformed cells for protein isolation.

Furthermore, Gomord et al. do not disclose or suggest that their approach may be scaled up in liquid culture to provide for protein isolation and characterization. To assume that it would be simple for one "skilled in the art" to adapt Gomord et al.'s 1 mL solid culture to large scale liquid culture is incorrect. Agrobacterium grows quickly in liquid culture and if one were to follow Gomord et al.'s protocol to scale up the culture for transient expression the procedure would fail because the Agrobacterium would rapidly grow and kill the cells.

Accordingly, the rejection of claims 1-3, 5-7, 11, 16 and 18 under 35 U.S.C § 102(b) as being anticipated by Gomord et al. is respectfully traversed.

IV. Rejection of Claims 1-3, 5-8, 11-12, and 15-20 Under 35 U.S.C § 103(a)

Claims 1-2, 5-8, 11, 12 and 15-20 are rejected under 35 U.S.C § 103(a) as being unpatentably obvious over Gomord et al. and Hiei et al. The Examiner states that Gomord et al. teaches a method for inoculating plant cells with Agrobacterium and suggests the use of the method to produce a variety of heterologous proteins. The Examiner relies on Hiei as teaching the use of acetosyringigone to pre-culture Agrobacterium for inoculation of monocots. The Examiner concludes, therefore, that it would have been obvious for one of ordinary skill in the art to utilize Gomord's method and to modify that method as taught by Hiei.

Applicant respectfully disagrees with the examiner's conclusion.

The discussion of Gomord et al. above is incorporated herein. As with the primary reference, Hiei et al. similarly discloses only stable transformation of plant cells. Hiei et al. discloses a procedure for the stable transformation of monocots. Like the Gomord et

al. procedure, Hiei et al. plates the cells on solid medium. Col.18: 60 through col. 19: 3. There is no discussion or suggestion of transforming plant cells in liquid medium and continuing liquid culture conditions. As such, this combination of references does not disclose or suggest the present invention.

Accordingly, the rejection of claims 1-2, 5-8, 11, 12 and 15-20 under 35 U.S.C § 103(a) as being unpatentably obvious over Gomord et al. and Hiei et al. is respectfully traversed.

V. Rejection of Claims 1-3, 5-7, 9-11 and 15-18 Under 35 U.S.C § 103(a)

Claims 1-3, 5-7, 9-11 and 15-18 are rejected under 35 U.S.C § 103(a) as being unpatentably obvious over Gomord et al. in view of Sastry et al. The Examiner relies on Gomord et al. as above. Sastry et al. is relied on as teaching the advantages of using an auxotrophic Agrobacterium. The Examiner states that the claims are broadly drawn to use of an auxotrophic Agrobacterium for inoculation and transformation of a liquid suspension of plant cells. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to use the method of the primary reference and to modify that method as taught by Sastry, given the broad applicability of the technique as suggested by Gomord.

Applicant respectfully disagrees with the Examiner's conclusion.

It is respectfully pointed out that claims 1-3, 5-7 and 15-18 are not limited by the genotype of the Agrobacteria used to inoculate the suspension of plant cells. Since the Examiner relies on Sastry as teaching use of an auxotrophic Agrobacterium, it appears that this rejection is applicable to claims 9-11, at best.

As discussed above, Gomord et al. only teaches stable transformation of plant cells for the isolation of heterologous proteins. There is no disclosure or suggestion of the use of liquid culture to obtain large scale transient transformation and isolation of heterologous protein. Moreover, the "broad applicability" of the procedure disclosed in Gomord is limited to the "generation of stably transformed" plant cells for studying various aspect of heterologous recombinant proteins. (Gomord et al., p. 156).

Sastry describes the use of auxotrophs for the purpose of attenuating tumorgenicity, e.g., **reducing the ability of the bacteria to transfer and integrate T-DNA** in the absence of supplemented nutrients. Thus, according to Sastry, if the Agrobacterium used in these studies were to escape from the lab (Petri-dish) environment, it would not have the media supplement and will no longer be able to transfer DNA. However, Sastry does not teach or suggest the use of auxotrophic Agrobacterium to attenuate and control growth of the bacteria in the bioreactor, thus ensuring that the bacteria do not overrun and kill the plant cells. For that matter, the tryptophan auxothrophs described by Sastry will not work for the purpose of controlled growth with plant tissues because of the tendency of plant cells to secrete phenolics. As such, the combination of this reference with Gomord et al. does not suggest the method of claims 9-11.

Accordingly, the rejection of claims 1-3, 5-7, 9-11 and 15-18 under 35 U.S.C § 103(a) over Gomord et al. in view of Sastry et al. is respectfully traversed.

VI. Rejection of Claims 1,2, 4-7, 11, 12 and 15-18 Under 35 U.S.C § 103(a)

Claims 1, 2, 4-7, 11, 12 and 15-18 stand rejected under 35 U.S.C § 103(a) as being unpatentably obvious over Gomord et al. in view of Wongsamuth et al. The Examiner relies on Gomord et al. as above. Wongsamuth et al. is relied on as teaching the use of *A. rhizogenes* to produce hairy roots for plant tissue culture mediated production of heterologous protein, including antibodies. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to use Gomord's method for producing heterologous proteins **in liquid culture** and to modify that method by incorporating *A. rhizogenes* strains and antibody encoding genes as taught by Wongsamuth.

Applicant respectfully disagrees with the Examiner's conclusion.

As discussed above, Gomord et al. does not disclose or suggest liquid culturing and transformation of plant cell, nor does this reference teach recovery of protein from transiently transformed liquid cultures of plant cells after a short period of growth. The secondary reference also fails to teach or suggest liquid transient transformation and isolation of large amounts of heterologous recombinant protein.

Wongsamuth teaches a process of stable genetic transformation, selection of a cell line and subsequent growth of that cell line to produce a protein. However, as discussed above, transient expression is significantly different from the general approach of transgenic plant or cell-line development taught by Wongsamuth. Thus, the combination of Gomord and Wongsamuth fails to disclose or suggest the present invention.

Accordingly, the rejection of claims 1, 2, 4-7, 11, 12 and 15-18 under 35 U.S.C § 103(a) as being unpatentably obvious over Gomord et al. in view of Wongsamuth et al. is respectfully traversed.

VII. Rejection of Claims 1-18 Under 35 U.S.C § 103(a)

Claims 1-18 are rejected under 35 U.S.C § 103(a) as being unpatentably obvious over Goodman. The Examiner states the cited prior art reference teaches production of polypeptides in plants and asserts that the sequence of steps in such method is an obvious design choice.

This rejection is respectfully traversed as follows.

Goodman et al. merely teaches transformation of plants using Agrobacteria as vector to stably transform plants or leaf discs, which are then grown under conditions to produce transformed plants. Goodman et al. does not teach co-culturing of plant cells/tissue with Agrobacteria, followed by liquid culturing of the co-culture for about one to four days, nor is there any disclosure or suggestion that recombinant polypeptide is obtainable from such co-culture. More particularly, Goodman et al. does not teach large scale co-culture of plant cells and/or plant tissue.

Furthermore, this reference neither discloses, nor suggests the method of the present invention in which plant cells or tissue are grown in liquid culture or grown and introduced to bioreactor culture conditions, infected with Agrobacteria and then grown in liquid culture for about one to four days under conditions favoring transient expression of recombinant polypeptide. In particular, Goodman et al. does not teach monitoring conditions during the a liquid culturing process in order to determine optimal conditions

for plant cell growth, timing of inoculation, or optimal conditions for gene transfer and transient expression of the recombinant polypeptide. Goodman et al. simply does not teach or suggest the conditions for cell growth or inoculation that are set forth in the claims and does not teach or suggest use of an auxotrophic Agrobacteria to control bacterial cell growth during production as set forth in the dependent claims. More particularly, Goodman et al. does not teach bioreactor culturing or liquid culturing of transiently transformed plant cells. As such, the cited reference does not render the present application obvious.

Accordingly, the rejection of claims 1-18 under 35 U.S.C. § 103(a) over Goodman et al. is respectfully traversed.

VIII. Rejection of Claims 1, 13, 19 and 20 Under 35 U.S.C. § 103(a)

Claims 1, 13, 19 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentably obvious over Goodman et al. in view of Baszczynski et al. The Examiner states that it is an inherent property of the claimed invention that the vector is stably incorporated into the plant genome and also asserts that transient expression is an inherent feature of Agrobacterial transformation of plant cells. Thus, the Examiner asserts that Goodman et al. teaches transient transformation of plant cells. The Examiner concludes, therefore, that the combined prior art teaches all of the limitations of the claimed invention.

Applicants respectfully disagree with the Examiner's conclusion.

The present invention is directed to a method of transiently transforming plant cells for large scale production of recombinant polypeptide within a short time frame, i.e.,

within about one to four days after transient transformation. In the present invention plant cells are grown in liquid media, inoculated with Agrobacteria and grown for about one to four days under conditions that enable transient expression of recombinant polypeptide in liquid culture. The culture is monitored to determine optimal growth conditions and timing for inoculation and harvesting of the recombinant polypeptide from the liquid co-culture.

In contrast, Goodman et al. and Baszczynski et al. both teach the necessity for transferring transformed plant cells to a medium suitable for growth of a stably transformed callus and other plant tissues after killing the majority of the other plant tissues that are not stably transformed. These references do not teach or suggest liquid cultures of transiently transformed plant cells for the production and recovery of recombinant polypeptide.

It is irrelevant that transient expression may occur (although this has not been demonstrated) during the processes disclosed by these two prior art references. It is also irrelevant that some stable transformation may occur during the claimed process (although this has not been demonstrated) for the simple reason that the prior art processes and the present invention are directed to very different plant transformation, culturing and harvesting processes. For example, Baszczynski teaches a method of plant transformation that utilizes viruses having a circular genome or replication intermediate (col. 5, line 60) and a recombinase in order to create a modified viral replicon.

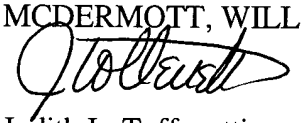
There is nothing in either of the cited prior art references that suggests liquid culture of Agrobacteria-transformed plant cells, much less a large scale production and recovery in a relatively short period of time. In fact, these references teach away from

liquid culturing of transformed plant cells. As such, the cited combination of prior art fails to render the present invention obvious.

Accordingly, the rejection of claims 1, 13, 19 and 20 under 35 U.S.C. § 103(a) over the cited prior art is respectfully traversed.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,
MCDERMOTT, WILL & EMERY



Judith L. Toffenetti
Reg. No. 39,048

600 13th Street, N.W.
Washington, DC 20005-3096
(202) 756-8000 JLT:men
Facsimile: (202) 756-8087
Date: July 10, 2003